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Invasive Aspergillosis in Transplant Recipients

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Abstract

Patients with hematological malignancies and recipients of allogeneic hematopoietic stem cell transplantation (allo-HSCT) as well as solid organ transplant recipients are the groups of patients with the highest risk of invasive fungal infections (IFI). Neutropenia, lymphopenia, chemotherapy of malignancies, radiation therapy, immunosuppressive therapy, administration of glucocorticosteroids, use of central venous catheters, dialysis therapy, liver and kidney failure, and diabetes are diseases and medical conditions which foster invasive fungal infections. In recent years, it has been observed that the most common etiological agents of these infections are yeast-like fungi of the genus *Candida*, and the second most common is moulds *Aspergillus* spp. Antifungal agent recommended for therapy of IFI caused by *Aspergillus* is voriconazole, according to the present guidelines. A combined therapy using voriconazole and caspofungin may not be effective. According to numerous publications, in case of infections caused by strains resistant to voriconazole, a therapeutic success is possible after a switch to the liposomal form of amphotericin B. Due to nonspecific clinical symptoms of IFI caused by *Aspergillus* spp., histopathological as well as mycological and serological tests, echocardiographic examination, magnetic resonance imaging (MRI) and computer tomography (CT) may contribute to an early and reliable diagnosis of invasive aspergillosis.

Keywords: *Aspergillus fumigatus*, invasive fungal infection, risk factors, antifungal therapy

1. Introduction

Transplant recipients constitute a group of patients who are immunocompromised. Among them, hematopoietic stem cell transplant (HSCT) recipients suffer from the most severe immunosuppression, which may be prolonged. Many risk factors make these patients prone to fungal infections caused by yeast-like fungi or molds.

Filamentous fungi of the genus *Aspergillus* may cause many clinical forms of the disease in immunocompromised patients (including HSCT recipients and solid organ transplant recipients), but increasingly also in patients undergoing intensive care or even without any immune deficiencies [1]. Aspergillosis usually results from inhalation of spores, affecting primarily the respiratory system [1–3]. Humans are exposed daily to massive numbers of fungal spores; however, usually they are eliminated by various pulmonary defense mechanisms, for example, mucociliary function [1, 2]. With progress in molecular diagnostic methods, particularly

next-generation sequencing (NGS) techniques, we have learned that molds and yeast-like fungi are present in the respiratory tract, even in healthy individuals [4]. Similar to microbiota composition in other parts of the body, the lung mycobiota also comprises numerous fungal species, which become less diverse in many diseases [4].

Aspergillosis may present in different forms, such as invasive aspergillosis (IA), allergic aspergillosis, chronic pulmonary aspergillosis, and as superficial disease in various anatomical locations (keratitis, otomycosis, and wound infections) [1, 4–6]. Allergic aspergillosis may present as allergic bronchopulmonary aspergillosis (ABPA) and allergic fungal rhinosinusitis (AFRS) [7].

Invasive aspergillosis is further divided into invasive pulmonary aspergillosis (IPA), sinusitis caused by *Aspergillus* spp., disseminated aspergillosis, and several types of invasive aspergillosis with the involvement of single organs [5, 6]. In transplant recipients and other immunocompromised patients, the most common clinical form of aspergillosis is invasive pulmonary aspergillosis, which untreated or unsuccessfully treated can lead to systemic dissemination to other organs and systems, for example, brain, heart, or the bones [1, 2]. In contrast, paranasal sinuses, larynx, eyes, ears, and the oral cavity are often involved in primary aspergillosis [2].

Invasive aspergillosis is the most common mold infection, particularly among immunocompromised patients. Patients subjected to allogeneic hematopoietic stem cell transplantation (alloHSCT) constitute a group of patients with the highest risk of systemic fungal infections, caused by both *Candida* spp., as well as *Aspergillus* spp. [5, 6]. Factors predisposing to the invasive aspergillosis are prolonged neutropenia ($<500/\mu\text{l}$ for >10 days) and lymphopenia (mainly affecting CD4⁺ cells) [5, 8–12]. Frequency of invasive infections caused by *Aspergillus* spp. is on the increase in patients undergoing chemo- and/or radiotherapy, treated with corticosteroids or immunosuppressive agents, as well as in patients with acquired immune deficiency syndrome (AIDS) or congenital deficiencies of the immune system, such as chronic granulomatous disease [1, 8, 10, 12–14]. Patients at the extremes of age (>60 and neonates), persons with chronic malnutrition, and individuals on total parenteral nutrition have fungal infections more often than patients in other groups [8, 10, 13]. Surgical procedures, particularly thoracic or abdominal surgery and solid organ transplantation, and the use of central venous catheters or dialysis catheters are linked to endogenous and exogenous infections, including those caused by *Aspergillus* spp. [1, 5, 6, 8–11]. Underlying diseases, such as diabetes, kidney, and/or liver failure, and chronic obstructive pulmonary disease (COPD) also predispose to invasive fungal infections [1, 8].

2. Etiology of invasive aspergillosis

Aspergillosis is caused by opportunistic molds of the genus *Aspergillus*, which contains more than 300 species; however, only relatively few of them are known to cause human diseases [12]. These fungi are ubiquitous in soil, plants, and decaying organic debris, as well as in household dust and building materials [1]. Hospitalized immunocompromised patients are at risk of aspergillosis during construction or renovation works at the hospital. Fungi *Aspergillus* spp. (like other molds), produce conidia that are easily aerosolized [12]. Inhaled conidia colonize the respiratory system of the host in whom—depending on the degree of immunosuppression—various clinical forms of aspergillosis may develop. Rarely, aspergillosis results from ingestion of the spores or their direct inoculation into the wounds [12].

The most common etiological agent of invasive aspergillosis with a high morality is *A. fumigatus*, responsible for the majority (up to 90%) of cases in humans [1, 12, 15–19]. It is followed by *A. flavus*, which causes up to 10% of cases of

bronchoalveolar aspergillosis [18]. This species also is responsible for most cases of aspergillosis with sinus and skin involvement [1]. It appears that *A. flavus* survives better than other *Aspergillus* spp. in a dry and hot climate; therefore, it is mainly isolated in Asia, Middle East, and Africa [17]. Other species of the genus *Aspergillus*, such as *A. niger*, *A. nidulans*, *A. terreus*, *A. versicolor*, *A. calidoustus*, and *A. ustus* cause invasive infections less frequently; however, they are of importance in immunocompromised patients [12, 18, 20, 21]. Strains of *A. niger* colonize the respiratory tract and are etiological agents of most cases of external otitis [1].

According to the Prospective Antifungal Therapy Alliance® (PATH Alliance®) registry, in a cohort study of 960 cases of proven/probable IA, *A. fumigatus* (72.6%) was the most predominant species, followed by *A. flavus* (9.9%), *A. niger* (8.7%), and *A. terreus* (4.3%) [12]. Recently, an increasing frequency of infections caused by environmental species of *Aspergillus* (of unknown significance in medicine) is being reported [21].

Apart from *Aspergillus* species other than *A. fumigatus*, recently attention is being focused on the strains classified in the section Fumigati (*A. fumigatus* complex), for example, *Neosartorya udagawae* (*A. udagawae*) [18, 22]. They may cause similar diseases as *A. fumigatus sensu stricto*; however, duration of illness may be prolonged (even sevenfold) [22]. It appears that actual prevalence of these cases may be underestimated, as these strains are often misidentified because they cannot be distinguished from *A. fumigatus sensu stricto* by conventional morphological tests [18]. Moreover, the outcome of treatment of these infections may be unfavorable, as strains belonging to *A. fumigatus*-related species (the section Fumigati) often show some level of intrinsic resistance to azoles and other antifungal drugs, with the minimum inhibitory concentrations (MICs) of various antifungals for these isolates higher than those for *A. fumigatus*, which is usually susceptible to azoles [22]. However, in a recent multicenter prospective study, the rate of azole resistance among *A. fumigatus* isolates was relatively high—3.2%, out of which 78% were *A. fumigatus sensu stricto* with a mutation of the *cyp51A* gene, while the remaining 22% were the related species (*A. lentulus*, *A. thermomutatus*, and *A. udagawae*) [23].

In clinical practice *A. lentulus*, *A. udagawae*, *A. viridinutans*, and *A. thermomutatus* (*Neosartorya pseudofischeri*), *A. novofumigatus* and *A. hiratsukae* have been linked etiologically to such refractory cases of IA [18]. These *A. fumigatus* complex strains are characterized by lower virulence ascribed to a lower thermotolerance and different profiles of secondary metabolites with decreased production of mycotoxins, such as gliotoxin [18]. Definitive identification of these cryptic species requires specific sequencing analyses of the beta-tubulin or calmodulin genes, which are not readily available. Clinical microbiologists should, therefore, be aware of such cryptic species of *Aspergillus* and the methods of their differentiation from *A. fumigatus*—defect in sporulation, unusual susceptibility profile to antifungals, as well as multiplex PCR assays and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique [18].

3. Clinical forms of invasive aspergillosis

Molds of the genus *Aspergillus* may cause a wide variety of clinical entities, ranging from asymptomatic colonization, allergic bronchopulmonary aspergillosis, and chronic pulmonary aspergillosis to severe (even fulminant) invasive infections in almost every organ or system in the body of the host, including (but not limited to) the lungs, heart, central nervous system, and the sinuses [5, 18, 19, 24]. The most common clinical form of invasive diseases caused by *Aspergillus* spp. is pulmonary aspergillosis, which in about 50% of cases spreads to other anatomical locations, including the

brain, liver, kidneys, endocardium, bones, and gastrointestinal tract [8, 10, 13, 19, 25]. Aspergillosis may also affect the paranasal sinuses, the ear, or the eyeball [13].

The spectrum of aspergillosis is determined by the host's immune status (particularly severe and prolonged neutropenia) and the virulence of *Aspergillus* strain causing the infection [5, 6, 12, 13]. In immunocompetent persons, molds of the genus *Aspergillus* mainly cause allergic symptoms and chronic pulmonary aspergillosis, without invasion and destruction of the host's tissues [12]. In the chronic pulmonary aspergillosis, usually a preexisting pulmonary condition is observed, such as preformed cavity in the lung (resulting from tuberculosis, sarcoidosis, or other necrotising process), in which an aspergilloma (or a fungus ball) is being formed (chronic cavitary pulmonary aspergillosis) [12]. Allergic bronchopulmonary aspergillosis (ABPA) is a disease that arises from a hypersensitivity reaction to *Aspergillus* antigens and most often develops in patients who have asthma, atopy, or cystic fibrosis [8, 12, 26]. Another form of aspergillosis, characterized by a local invasion of the lung parenchyma; however, without invasion or dissemination to other organs, is called subacute invasive aspergillosis (or chronic necrotizing pulmonary aspergillosis) [12].

Invasive aspergillosis is a life-threatening infection and a major cause of death in immunocompromised patients, particularly hematopoietic stem cell transplant recipients, but may be fatal even in immunocompetent individuals, with the death rate of 40% for pulmonary disease, 90% for disseminated disease, and practically 100% for disseminated disease with the central nervous system (CNS) involvement [1, 19]. According to the literature, invasive aspergillosis occurs in 1–15% of the solid organ transplant recipients, in whom mortality rates due to this disease range from 65 to 92% [1]. However, currently these indices improve in these patients due to advances in immunosuppressive regimens and transplantation practices, as well as frequent use of antifungal prophylaxis.

Recent reports indicate that invasive aspergillosis is being increasingly diagnosed in patients without above-mentioned severe neutropenia—particularly in the lung transplant recipients, patients hospitalized in the intensive care unit or treated with corticosteroids [9, 11, 27]. Also, individuals with chronic obstructive pulmonary disease (COPD), liver failure, and other underlying diseases are listed in this group [8, 11, 20]. It has been reported that in ICU patients with invasive pulmonary aspergillosis 40–80% of them do not have any malignancy or other classical risk factors for this infection [8, 20]. Moreover, in contrast to patients with neutropenia, in ICU patients the symptoms of aspergillosis are atypical; therefore the diagnosis of this infection may be delayed or omitted, according to the autopsy studies [11, 15, 28]. Tejerina et al. showed that among 893 deceased patients, previously treated in ICU, only 40% (10 out of 25) had invasive aspergillosis diagnosed *ante mortem* [11]. Problems with proper diagnosis of invasive aspergillosis, and therefore a delay in administration of effective antifungal therapy, are undoubtedly linked to a high mortality rate in this group of patients (30–40%), which may even exceed 90% despite lack of severe immunosuppression in these individuals [15, 27, 29].

3.1 Aspergillosis of the oral cavity and the upper respiratory tract

As mentioned earlier, aspergillosis affects mainly the lungs; however it may also be diagnosed in the upper respiratory tract and the oral cavity [2, 30]. Orofacial aspergillosis is relatively common in oncohematological patients undergoing chemotherapy [2].

Aspergillosis of the larynx is very rare, with only a few cases reported in the literature [31]. Usually, it is secondary form of this disease, while primary aspergillosis of the larynx is even rarer. Risk factors for aspergillosis of the larynx comprise

the use of inhaled or systemic steroids, prolonged antibiotic therapy, and underlying immunosuppression [31]. It should be emphasized that these lesions may mimic malignancy or a premalignant condition, while proper diagnosis and administration of antifungal therapy, as well as elimination of the risk factors (if possible), are effective in the elimination of this condition [31].

It also appears that chronic tonsillitis may be caused by *Aspergillus* spp. In a study of 75 symptomatic children who underwent a tonsillectomy, in 9 (12%) of them *Aspergillus* was detected on histological examination of the removed tonsils [30].

Aspergillus spores may be deposited in the oral cavity upon inhalation or during dental procedures, for example, tooth extraction [3]. The fungus may then spread further into the sinuses as odontogenic infection [32, 33]. The infection may also become established in the oral cavity itself.

In oral aspergillosis, the lesions are usually located on the palate or posterior tongue [2]. They are yellow or black, with a necrotic ulcerated base. The hyphal elements of *Aspergillus* fungus may invade the oral mucosa through the release of various toxins, such as proteases, phospholipases, hemolysins, gliotoxin, aflatoxin, phthioic acid, and many others. Subsequently, they penetrate the blood vessels, producing thrombosis, infarction and necrosis, and then systemic spread follows [2].

3.2 Aspergillosis of the nose and paranasal sinuses

Paranasal sinuses may be colonized or infected by fungi, while infection can be invasive (acute or chronic) or noninvasive (allergic sinusitis and aspergilloma) [18]. In invasive aspergillosis of the sinuses, there are rapid (within a few days) destructions of the sinuses, the nasal cavity, and the adjacent structures, such as the orbit and the brain [18]. *Aspergillus* spp. may also cause allergic *Aspergillus* sinusitis (AAS), which results from a hypersensitivity reaction to the presence of the fungus in the sinus [26]. The hallmark of AAS is demonstration of fungal elements in the material obtained from the sinus [26].

It is estimated, that fungal sinusitis constitutes 6–9% of all cases of rhinosinusitis. Among fungi causing sinusitis, the most common is *Aspergillus* spp. [18]. Aspergillosis of the paranasal sinus should be suspected in patients with refractory or recurrent sinusitis. Apart from the sinuses, aspergillosis may also affect the nasal cavity, from which the infection can spread to the CNS (rhinocerebral aspergillosis).

The maxillary sinus is more often affected than other paranasal sinuses. However, it is frequently misdiagnosed, even as malignancy [3, 18, 34]. Untreated infection may spread to the other structures in the head [35]. Invasive aspergillosis of the maxillary sinus should be considered in patients with maxillary sinusitis which does not respond to standard therapy with antibiotics, even in immunocompetent patients [3].

From the paranasal sinuses, *Aspergillus* infection may spread locally into the vasculature and the brain, leading to cavernous sinus thrombosis and a variety of central nervous system manifestations and to other locations, for example, the orbit [36]. In these cases, computed tomography (CT) or magnetic resonance imaging (MRI) and biopsy of any lesion located in the sinuses or the nasal cavity in high-risk patients is mandatory [19]. Intracranial and intraorbital extension decreases the survival rate of these patients [3].

Factors which predispose immunocompetent individuals to fungal infections in the sinuses include polyps and blocked drainage of secretions [3]. Additional risk factors for fungal sinusitis, including *Aspergillus*, are reported in immunocompromised patients and individuals with various underlying diseases, such as neutropenia, immunosuppressive therapy, corticosteroids, uncontrolled diabetes mellitus,

trauma, burns, and radiation therapy [3]. In these patients, particularly with hematological malignancies and in transplant recipients, *Aspergillus* may cause an invasive infection as aggressive in its clinical course as those caused by *Zygomycetes* [19]. Invasive fungal sinusitis is potentially fatal, with an extremely high mortality rate, particularly in immunocompromised patients [3]. Therefore invasive aspergillosis of the paranasal sinuses has to be recognized and treated to avoid significant mortality in immunocompromised patients, particularly in transplant recipients [3]. It should be suspected in cases of purulent rhinosinusitis not responding to repeated courses of antibiotics, and on the basis of radiological features.

Therapy of fungal sinusitis depends on its clinical form. In cases of *Aspergillus* fungal ball of the paranasal sinuses, surgical removal alone can be sufficient [5, 6]. Enlargement of the sinus opening may be needed to improve drainage and prevent further recurrence of sinusitis [5, 6]. In invasive aspergillosis apart from surgery, also systemic antifungal therapy is recommended.

3.3 Aspergillosis of the lower respiratory tract

Within the lower respiratory tract, *Aspergillus* infection may affect the larynx, trachea, and bronchi (tracheobronchitis), as well as lung parenchyma (pulmonary aspergillosis). In rare cases, *Aspergillus* pleuritis has been reported [19].

The lungs are affected in up to 92% of all cases of invasive aspergillosis [19]. Invasive pulmonary aspergillosis is a clinical entity characterized by invasion of the fungal hyphae into the blood vessels (angioinvasion), which subsequent hemoptysis. Other symptoms comprise nonproductive cough, pleuritic pain, low-grade fever, and dyspnea [19]. However, in the early stages of the disease, both clinical symptoms and radiological findings may be nonspecific, so proper diagnosis and treatment may be delayed, resulting in increased mortality in this group of patients.

The frequency of invasive pulmonary aspergillosis has significantly increased in recent years due to a growing number of immunocompromised patients [8, 37]. This clinical entity most often occurs in patients with different forms of immunosuppression, for example, hematological malignancies, profound and prolonged neutropenia, or corticosteroid therapy, as well as organ transplantation, autoimmune and inflammatory conditions, in critically ill patients, and those with chronic obstructive pulmonary disease (COPD) [8, 37]. In about 50% of patients with invasive pulmonary aspergillosis, the infection spreads to other anatomical sites, such as the brain, liver, kidneys, or the gastrointestinal tract [8, 10, 13, 19, 25].

An uncommon clinical form of IA is an isolated invasive *Aspergillus* tracheobronchitis (iIATB), in which fungal infection is limited predominantly or entirely to the tracheobronchial tree [19, 38]. It has been mainly reported in lung- and heart-lung transplant recipients. Wu et al. reviewed 19 cases of this disease and concluded that iIATB occurs in moderately or nonimmunocompromised patients with impaired airway structures or defense functions and it may be an early period of invasive pulmonary aspergillosis [38]. Early diagnosis and effective antifungal treatment were linked to a favorable prognosis; however, 5 out of 19 (26.3%) patients died.

Other forms of *Aspergillus* infection of the lungs comprise chronic necrotizing aspergillosis, which is described in patients with chronic lung disease or low degree immunodeficiency as a locally invasive disease, as well as aspergilloma usually found in individuals with previously formed cavities in the lungs [8, 12].

As mentioned earlier, symptoms of the pulmonary disease may result not from actual infection, but allergic reaction of the host to the presence of *Aspergillus* in the bronchial tree—allergic brochopulmonary aspergillosis (ABPA) [12, 26].

This immunologically mediated disease occurs predominantly in patients with asthma, atopy, and cystic fibrosis (CF) [8, 12, 26].

3.4 Aspergillosis of the central nervous system

Aspergillosis of the brain (cerebral aspergillosis) is usually a part of the disseminated disease after hematogenous spread of infection from the lungs, but rarely it may represent an isolated disease of the central nervous system (CNS) [19]. It is reported in 10–15% of patients with invasive pulmonary aspergillosis [39]. The most common risk factors comprise neutropenia and other forms of immunosuppression and transplantation surgery [19]. Cerebral aspergillosis usually presents as a single or multiple brain abscess, also as cerebral vasculitis and cerebral infarcts, while meningitis is rare [19]. Brain abscesses are common in HSCT recipients, while relatively rare in solid organ transplant (SOT) recipients [40, 41]. CT and MRI are recommended in patients in whom cerebral aspergillosis is suspected [42]. Among all types of IA, brain aspergillosis has the worst prognosis, with mortality rate usually exceeding 90% (up to 100%), however early and proper treatment improves the prognosis in these patients and significantly increases their survival rates [19].

Aspergillosis of the CNS may also present as rhinocerebral aspergillosis, particularly in patients with underlying malignancies and neutropenia, HSCT recipients, and patients with diabetic ketoacidosis [37]. Symptoms usually comprise fever, nasal or sinus congestion or pain, nasal discharge, unilateral facial swelling, and headaches [37]. Necrotic lesions may be seen on the hard palate and in the nasal cavity. The spread of infection may lead to ophthalmic complications, such as ptosis, proptosis, and vision disturbances [37].

3.5 Invasive cardiac aspergillosis

Invasive cardiac aspergillosis may present as endocarditis, myocarditis, pericarditis, mediastinitis, septic thrombophlebitis, and infections of aortic grafts—also in transplant recipients [5, 6, 43, 44].

Endocarditis caused by *Aspergillus* spp. is a severe form of fungal endocarditis [45, 46]. The mortality rate is high and surgery is usually required [19, 45–47]. It is very rare, but in recent years, the incidence of this form of aspergillosis is increasing, due to a rise in the frequency of its risk factors—the use of invasive procedures involving the heart, cardiac surgery with replacement of the heart valves, implantation of cardiac devices, organ transplantation, drug abuse, or administration of immunosuppressive therapy [19, 45]. Pierrotti and Baddour analyzed mold-related endocarditis in 3939 patients (the majority of cases were caused by *Aspergillus* spp.) and found the following risk factors: underlying cardiac abnormalities (41%), prosthetic heart valves (39%), malignancy (18%), solid-organ transplantation (18%), and bone marrow transplantation (18%) [47]. In a study by Paterson et al., 26% of cases of infective endocarditis occurring within a month of solid organ transplantation were caused by *Aspergillus* [48]. Woods et al. found that the most common predisposing factors for *Aspergillus* endocarditis in 29 patients were corticosteroid therapy (55%), prolonged antibiotic treatment (31%), hematological malignancy (28%), and chemotherapy and cytotoxic therapy (28%) [49].

In the course of *Aspergillus* endocarditis, mitral and bicuspid valves (native or prosthetic) are most often affected [19, 46, 50, 51]. Fungal vegetations may be formed on the heart valves, which subsequently may cause embolism blocking the arteries. Gumbo et al. reported that vegetations were revealed on the heart valves in 78% of the cases in which echocardiography was performed, while embolic

episodes were seen in 69% of patients with *Aspergillus* endocarditis, and a new or changing heart murmur—in 41% of them [52]. In a recently published study, Meshaal et al. showed that aortic abscess or pseudoaneurysm was one of the strong predictors of *Aspergillus* endocarditis [45]. However, it should be noted that *Aspergillus* endocarditis may be difficult to diagnose because blood cultures are usually negative, while fever is absent in 26% of patients with this disease [19, 45]. *Aspergillus* endocarditis should, therefore, be suspected in patients with underlying immunosuppression, hematological malignancies, recent cardiothoracic surgery, intravenous drug use, systemic or pulmonary emboli with negative blood cultures, and vegetation on echocardiography [24]. Diagnosis should be confirmed by histology and mycological culture of tissue or vegetation samples [24]. Delayed or erroneous diagnosis of *Aspergillus* endocarditis contributes to incorrect management of patients. Early surgical replacement of an infected valve and prolonged administration of antifungal therapy (which may be lifelong) are recommended to prevent embolic complications, valvular decompensation, and further spread of the infection [5, 6, 24, 43, 44].

Heart muscle may be affected by *Aspergillus* infection in the form of myocarditis or cardiomyopathy [53, 54]. This form of disease usually results from hematogenous spread of infection and is characterized by the presence of abscesses. Pericarditis caused by *Aspergillus* spp. has been described, also in transplant recipients [43, 55].

3.6 Ocular aspergillosis

Aspergillosis of the eye may take a form of fungal keratitis, endophthalmitis, or invasive aspergillosis of the orbit. All structures of the eyeball may be affected—eyelids, conjunctivae, lacrimal apparatus, cornea, sclera, or uvea. *Aspergillus* dacryocystitis is a rare complication of aspergillosis of the paranasal sinuses [56]. Clinicians must be aware that the clinical course of ocular aspergillosis may range from asymptomatic infection or slowly developing disease to rapidly progressive infection, with a fulminant course and fatal outcome [56].

Fungal keratitis often presents as a corneal ulcer, which results from mechanical injury of the cornea, with subsequent necrosis. In a recent study, Manikandan et al. examined a total of 500 corneal scrapings, collected from patients in whom mycotic keratitis was suspected, out of which 68 (13.6%) were positive for *Aspergillus* spp. [57].

Endophthalmitis may be a complication of surgical procedures or may result from hematogenous spread from other sites of infection. Endogenous *Aspergillus* endophthalmitis is mainly reported in severely immunocompromised patients, transplant organ recipients, patients after heart valve replacement, and in oncohematological patients [58–60].

Orbital invasive aspergillosis is a rare infection, which most often results from dissemination of the infection from the nose or paranasal sinuses. It may take an acute or chronic form. This form of IA usually presents with a severe orbital pain, paralysis of the oculomotor nerve and visual impairment. Ophthalmic complications, such as ptosis, proptosis, and vision disturbances may result from the spread of infection in rhinocerebral aspergillosis [37].

3.7 Aspergillosis of the genitourinary tract

Aspergillosis of the urinary tract is relatively rare. In immunocompromised patients, such as kidney transplant recipients, frequency of this form of aspergillosis amounts to only 0.5–2.2%, but is fatal in >88% of patients [61]. Urinary tract aspergillosis may also be a complication of surgical procedures, such as lithotripsy,

urinary tract instrumentation, or ureteric stenting, particularly in immunocompromised patients [62–65]. Renal aspergillosis is being occasionally reported with obstruction of one or both ureters [19]. Localized aspergillosis of the renal graft was reported in a child 5 months after kidney transplantation [61]. A case of urinary tract aspergillosis presenting as an aspergilloma of the urinary bladder has also been described [66].

Aspergillosis of the genital tract has been rarely reported [67–69]. In women, it may give the symptoms resembling a pelvic inflammatory disease.

3.8 Other clinical forms of aspergillosis

Other clinical forms of aspergillosis—apart from above-mentioned—are very rare and comprise gastrointestinal aspergillosis, cutaneous aspergillosis, *Aspergillus* peritonitis, osteomyelitis, and septic arthritis, as well as *Aspergillus* ear infections [5, 6, 19, 37].

Gastrointestinal aspergillosis is mainly reported in patients who are transplant recipients or severely malnourished [37]. It results from ingestion of *Aspergillus* spp. spores. The most commonly involved sites are the stomach, colon, and ileum.

Cutaneous aspergillosis is classified as primary or secondary [37]. Primary form of the disease is usually reported in patients with burn wounds or surgical wounds. This clinical entity is caused by direct inoculation of the fungus or its spores into the injured skin [37]. Primary cutaneous aspergillosis has been linked to leukemic patients, neonates, transplant recipients, as well as the use of occlusive dressings or permanent intravenous catheters [19]. Secondary cutaneous aspergillosis results from hematogenous spread of the fungus and therefore represents a disseminated form of *Aspergillus* infection. It may present as nodules or extensive necrotic lesions [19]. The mortality rate may be high.

Aspergillus peritonitis is seen in patients undergoing peritoneal dialysis [19]. It may be complicated by hemorrhage, perforation, or infarction.

Aspergillus ear infections may present as noninvasive otitis externa (otomycosis) or invasive aspergillosis of the ear. Examples of other forms of invasive aspergillosis comprise vertebral osteomyelitis (resulting from hematogenous spread, by contiguity or from direct inoculation), septic arthritis, cholangitis, and prosthetic vascular graft rejection [19].

Disseminated aspergillosis is defined as the involvement of at least two noncontiguous sites in the body, mainly in transplant recipients [19]. Disseminated disease has been reported in 9–36% of kidney transplant recipients, 15–20% of lung transplant recipients, 20–35% of heart transplant recipients, and 50–60% of liver transplant recipients with invasive aspergillosis [70].

4. Aspergillosis in transplant recipients

In patients with immune deficiencies and other risk factors, invasive aspergillosis (IA) is a life-threatening infection caused by the opportunistic molds of the genus *Aspergillus*, most often by *A. fumigatus* [5, 6, 14, 70, 71]. A cohort study of 960 cases of probable or proven IA reported in the Prospective Antifungal Therapy Alliance (PATH Alliance) registry, indicated that 48.3% of patients had hematologic malignancy, 29.2% were SOT recipients, 27.9% were HSCT recipients, and 33.8% were neutropenic [12]. In these patients, the most common clinical forms of IA are invasive pulmonary aspergillosis (IPA) and rhinocerebral aspergillosis [12].

In transplant recipients, particularly in hematopoietic stem cell transplant (HSCT) recipients who are severely immunocompromised, invasive aspergillosis is not only more common than in other groups of patients, but is also characterized by

much more severe and rapidly progressive clinical course, and much higher mortality [12, 72].

Although prognosis has improved in recent years, IA still remains a significant post-transplant complication in solid organ transplant (SOT) recipients [43, 44]. It occurs in up to 30% of SOT recipients. Although in recent years the mortality rate in transplant recipients decreased from 65–92% to 22%, still an estimated 9.3–16.9% of all deaths in transplant recipients in the first year after transplantation can be attributed to IA [44].

The incidence of IA differs between different populations of transplant recipients, and unique risk factors for *Aspergillus* infections have been identified for various types of organ transplant recipients [43, 44]. However, regardless of the type of transplantation a major risk factor for the development of IA in SOT recipients is the net state of immunosuppression including the intensity of administered immunosuppressive therapy [44].

Regarding the risk of invasive fungal infections (particularly IA) in transplant recipients, at present routine antifungal prophylaxis or preemptive therapy is recommended in HSCT recipients with prolonged neutropenia or graft-versus-host disease (GVHD), and in lung transplant recipients, while targeted prophylaxis should be considered in liver and heart transplant recipients [5, 6, 44, 71].

4.1 Hematopoietic stem cell transplant (HSCT) recipients

Invasive aspergillosis remains a major complication following allogeneic HSCT (alloHSCT) [73]. The burden of IA has increased significantly in the last 30 years as a result of the increased number of patients undergoing immunosuppressive therapy for hematological malignancies and HSCT [14]. It is estimated that IA is a leading cause of fatal outcomes in HSCT patients, accounting for 10% of all deaths in this group of patients [14].

In the recipients of alloHSCT, IA can occur during the neutropenic pre-engraftment phase (early IA) or during the post-engraftment period (late IA) [14]. Although IA remains an important complication after allogeneic transplantation, regardless of the type of conditioning regimen, early IA is more common in patients undergoing myeloablative transplantation due to extensive chemotherapy and radiation used to destroy the native bone marrow, and prolonged neutropenia which results from this treatment [14, 74]. On the other hand, non-myeloablative HSCT comprises a shorter neutropenic period and therefore, a decrease in the incidence of early IA. However, a higher risk of GVHD in this group of patients and the therapies given for this condition caused a shift to late IA, diagnosed increasingly during the post-engraftment period [14]. It should be noted that the risk period for late IA, associated with GVHD, can last for months to years, so prophylactic and monitoring procedures must be implemented over a long period. Carvalho-Dias et al. analyzed 24 cases of proven and probable invasive aspergillosis among HSCT recipients and reported, that 83% of the patients died due to invasive fungal infection within 60 days of follow-up [72].

Kojima et al. compared the incidence of IA and mortality rates due to this disease in 664 recipients of alloHSCT—486 conventional stem cell transplantation (CST) patients and 178 reduced-intensity stem cell transplant (RIST) recipients. The overall incidence of IA in all 664 recipients of alloHSCT was 35 (5.3%) [74]. Despite significant differences in the estimated 3-year incidence of IA in CST group (4.5%) and RIST population (8.2%) ($P = 0.045$), the mortality rates were similar in both groups (76 and 86%, respectively). However, the median onset of IA after RIST was day 127, which was significantly later than that after CST—day 97 [74]. Furthermore, a multivariate analysis showed that IA was associated with

age > 50 years and the presence of acute or chronic GVHD [74]. In another study, Labbe et al. analyzed the risk factors for IA in 125 alloHSCT recipients with nonmyeloablative (NMA) regimens, who received a 6/6 matched sibling NMA HSCT and were treated homogenously [73]. IA developed in 13 patients (5 proved, 6 probable, and 2 possible IA), at 44–791 days (median 229 days) after NMA HSCT. The risk of IA was calculated as 7% at 1 year, 11% at 2 years, and 15% at 3 years after NMA alloHSCT [73]. It was concluded that in NMA HSCT recipients the risk of IA increases over time and is significantly associated with intestinal GVHD, therefore these patients should be monitored for this complication and administration of antifungal prophylaxis with activity against molds should be considered [73].

4.2 Lung and heart-lung transplant recipients

In lung transplant recipients, invasive aspergillosis is the predominant fungal infection [44, 71, 75]. In the past, the incidence of IA in this group of patients was reported in the range of 4.0–23.3%, however, newer studies point to a lower frequency of this disease [44]. On the other hand, the time from lung transplantation to the onset of IA becomes longer due to the use of antifungal prophylaxis in the early post-transplant period. Therefore the median time to IA onset has increased from 120 days post-transplant to 483 or 508 days post-transplant reported in recent studies [44].

The most common species linked to the etiology of IA in lung transplant recipients is *Aspergillus fumigatus*. In an international, multicenter, retrospective cohort study of 900 consecutive adult lung transplant recipients with 4 years of follow-up, 79 patients developed 115 episodes of IA [75]. *Aspergillus fumigatus* was isolated in 72 of 115 (63%) episodes [75]. In a retrospective study of 251 lung transplant recipients, *Aspergillus* was isolated from 86 (33%) cases including 50 patients colonized with *Aspergillus* spp., 17 recipients with tracheobronchitis, and 19 cases of IA [76]. These authors reported that isolation of *Aspergillus* spp. from respiratory samples preceded acute rejection of the graft, therefore it may be a marker of threatening graft rejection and/or inflammation of the airways [76].

The significance of the patient's airway colonization with *Aspergillus* spp. before lung transplantation remains controversial. Some authors indicate its importance within the first year after lung transplantation, if the recipient was colonized in the period of 6 months before lung transplantation, while others did not find any significant link between *Aspergillus* pre-transplant colonization and occurrence of IA in the post-transplant period [44, 75–77]. Other risk factors for IA, which are unique to lung transplant recipients, comprise bronchial anastomotic leaks and other complications within the surgical site, airway narrowing, allograft dysfunction and/or graft ischaemia, reperfusion injury, CMV infection, bronchiolitis, and requirement for more intensive immunosuppressive therapy to prevent graft rejection [44, 76]. In lung transplant recipients, recovery of *Aspergillus* spp. from a respiratory tract sample warrants bronchoscopy to exclude the presence of tracheobronchitis [44].

It should be beared in mind that in lung transplant recipients there is a continuous exposure of the graft to the external environment through the airways, with impaired defense mechanisms (decreased mucociliary function, weakened cough reflex) in the early postransplant period [44].

In lung transplant recipients, there is a transient devascularization of the bronchial anastomotic site, which may contribute to ischemic injury and necrosis. This is a risk factor for development of ulcerative tracheobronchitis—a locally invasive form of IA involving the anastomotic site, the trachea, and the bronchi [44, 78]. In these patients, bronchovascular fistulas may develop, with a potentially fatal hemorrhage.

In the literature reports, the mortality rate of lung transplant recipients with IA ranges from 23 to 29% in individuals with tracheobronchitis to as high as 67–82% in patients with invasive pulmonary aspergillosis, but according to some estimates at present, it could be as low as 20% [44]. In a follow-up study of 251 lung transplant recipients, *Aspergillus* infection was associated with a reduced 5-year survival rate of these patients [76]. Prognosis is worse in patients with aspergillosis of the central nervous system or with disseminated disease [71].

4.3 Heart transplant recipients

According to the literature, the incidence of invasive aspergillosis in heart transplant recipients ranges from 1 to 14% [44, 79]. In this population, the risk factors for IA comprise isolation of *Aspergillus fumigatus* from bronchoalveolar lavage fluid, disease caused by cytomegalovirus, reoperation, and post-transplant hemodialysis [44]. The mortality rate in heart transplant recipients with invasive aspergillosis remains high—in the range of 66–88% [44, 79–81].

Invasive pulmonary aspergillosis remains the most common clinical presentation of this disease, particularly in early-onset IA (≤ 3 months after transplantation), while in late-onset IA, there is a higher frequency of disseminated disease and involvement of the central nervous system and other extrapulmonary sites [79, 80]. In an analysis of 455 heart transplant recipients, in whom 8 cases of IA have been diagnosed, all had invasive pulmonary form of the disease [79]. Risk factors for early-onset IA (within ≤ 3 months after heart transplantation), comprised hemodialysis, thoracic reoperation, and the presence of another case in the institution within the preceding 3 months [79]. For late-onset IA in this population of heart transplant recipients, hemodialysis and augmented immunosuppression were identified as risk factors [79]. In the clinical course of these cases, predominated septic shock and multiple organ dysfunction syndrome (MODS), nonspecific clinical and radiographic findings, as well as rapid (at a median of 11 days after diagnosis) mortality despite administration of antifungal therapy with activity against molds [79]. In a study by Montoya et al., none of the heart transplant recipients with either invasive pulmonary aspergillosis or invasive extrapulmonary aspergillosis had neutropenia [81]. Therefore, even in the absence of neutropenia invasive pulmonary aspergillosis should be suspected, particularly within the first 3 months of transplantation in heart transplant recipients who have fever and respiratory symptoms, a positive result of culture of respiratory secretions, and abnormal radiological findings (particularly nodules) [81].

A study of 479 consecutive heart transplant recipients in a single institution revealed a decrease in the incidence of IA from 8.7% (24/277) in the period 1988–2000 to 3.5% (7/202) in 2001–2011 [80]. Overall the incidence of IA in the studied group of heart transplant recipients was 6.5% (31 of 479). However, the authors report that four of seven cases were diagnosed as an outbreak, which indicates that favorable conditions for an infection with *Aspergillus* spp. may be present in a hospital [80]. Over the study period, there was a decrease in the mortality rate among the heart transplant recipients with IA from 46 to 0% ($p = 0.04$) [80]. The authors also noted a higher mortality rate in late-onset IA cases (63%) in comparison to early-onset IA (26%, $p = 0.09$) [80].

4.4 Liver transplant recipients

Invasive aspergillosis is reported in 1.0–9.2% of the liver transplant recipients. Mortality rates have decreased from 83–88% in earlier studies to 33–65% in more recent reports [44]. However, they remain very high in patients who develop invasive

aspergillosis after liver retransplantation (82.4%), particularly in those undergoing surgery later than 30 days after primary liver transplantation (100%) [44].

Risk factors for invasive fungal infections, including aspergillosis, in these patients, comprise retransplantation (30-fold higher risk) and renal dysfunction, particularly requiring any form of renal replacement therapy (15- to 25-fold higher risk) [44]. Furthermore, transplantation for fulminant hepatic failure, pretransplant corticosteroid therapy, cytomegalovirus (CMV) infection, and prolonged intensive unit care stay are other risk factors associated with invasive aspergillosis in liver transplant recipients [44]. It is underlined that liver transplant recipients are particularly susceptible to disseminated and central nervous system invasive aspergillosis [44].

Previously most invasive fungal infections in liver transplant recipients occurred within the first month after transplantation (the median time to onset was reported as 16–17 days), however, in recent years, they are usually diagnosed in the late period (>90 days) after liver transplantation. After renal replacement therapy and retransplantation, the median time to onset of IA was reported as 13 and 28 days, respectively [44].

4.5 Kidney transplant recipients

Invasive aspergillosis has been reported in 0.7–4.0% of the renal transplant recipients [44]. It should be emphasized that despite a relatively low overall incidence of IA in comparison to other solid organ transplant (SOT) recipients, the mortality rate is high in these patients—in the range of 67–75% [44]. Risk factors for invasive aspergillosis in kidney transplant recipients are the following: potent immunosuppressive therapy, leukopenia, prolonged and/or high dose corticosteroid therapy, longer duration of renal replacement therapy in the pretransplant period, and graft failure requiring hemodialysis [44, 82].

In a recent study, Desbois et al. analyzed the outcome of IA in kidney transplant recipients in the era of voriconazole availability [83]. Unfortunately, they concluded that the prognosis of patients with IA after renal transplantation is still poor, and even if the patients survive, the risk of graft loss is high [83].

5. Mycological diagnostics

The diagnosis of invasive aspergillosis remains a significant challenge [19]. It is usually based on a histopathological evidence of tissue invasion, in conjunction with an isolation of *Aspergillus* spp. in culture of the biopsy material or other clinically relevant specimen, as well as compatible clinical signs and symptoms in a patient with recognized risk factors. Imaging examinations (radiographic, computed tomography, and magnetic resonance), as well as serological tests (detection of fungal antigens and antifungal antibodies), provide only additional information and should be interpreted in conjunction with the clinical picture and the results of additional laboratory tests [13, 19, 48].

It should be emphasized that fast detection and identification of the etiological agent of infection is of utmost importance as it allows an early start of an effective antimicrobial therapy, which improves the patient's chances to survive.

In mycological diagnostics, the culture of the fungus from the site of infection and *in vitro* susceptibility testing of the isolate remain the “gold” standard. A diagnosis of invasive pulmonary aspergillosis comprises detection of the fungal mycelium with histopathological (with the use of different staining techniques) tests and/or positive culture of the relevant material obtained from the lower

respiratory tract [5, 6, 13, 15, 19]. In a patient in whom invasive pulmonary aspergillosis is suspected, it is recommended to culture a sample of bronchoalveolar lavage fluid (BALF), obtained as early as possible during the course of infection [5, 6, 13, 19]. It should be emphasized that the result of sputum culture is not an unequivocal proof of invasive infection of the lung parenchyma, because it may represent only colonization of the respiratory tract by the isolated microorganism. It has been reported that among 66 elderly patients in whom *Aspergillus* spp. was cultured from sputum, only 3 individuals had invasive pulmonary aspergillosis [84]. In another study, no *Aspergillus* spp. was cultured from the sputum of 70% of patients with invasive pulmonary aspergillosis confirmed with other reliable methods [8].

Blood cultures are rarely positive in patients with invasive pulmonary aspergillosis [8, 19, 45]. In patients with pulmonary aspergillosis, *Aspergillus* fungaemia was detected in 10.1% (9/89) of them, at a median of 5 days from the onset of clinical symptoms [19]. The diagnostic role of *Aspergillus* fungaemia in patients with an invasive form of infection is limited because blood cultures become positive (if at all) in the late stage of the disease when a microbiological or clinical diagnosis has already been made [19].

In laboratory diagnostics of this form of aspergillosis, detection of *Aspergillus* spp. antigen—galactomannan (GM)—in BALF or in serum, may be useful; however, it should be remembered that it could represent a false positive result [10, 13, 15, 19, 29]. Detection of another fungal antigen which is a constituent of the fungal cell wall—(1–3)- β -D-glucan (BDG)—is a nonspecific marker of fungal infection, being positive in many fungal infections apart from aspergillosis [13]. Molecular methods (e.g. detection of DNA of the strains classified in the genus *Aspergillus* in blood or BALF using PCR technique) are promising, however at present, they are not routinely available in clinical microbiology laboratories, and interpretation of their results requires further analyses [13, 19, 85]. Research is ongoing on the usefulness of serum interleukin-8 concentration as an auxiliary marker in laboratory diagnostics of invasive pulmonary aspergillosis [86]. Therefore, it should be emphasized that diagnosis of invasive pulmonary aspergillosis requires evaluation of the patient's clinical status in conjunction with the results of various examinations—imaging, histological, and mycological, as well as biochemical markers [1, 8, 13, 15, 86].

Laboratory methods currently used in diagnosis of invasive aspergillosis comprise three groups of techniques: detection of fungal invasion in histopathological examination of tissue sections; direct microscopy and isolation of *Aspergillus* spp. in culture of the clinically relevant samples; and noninvasive methods such as serological detection of antigens or nucleic material of *Aspergillus* spp., or detection of antibodies [19, 87].

5.1 Histopathological examination of tissue sections

Histopathological examination of biopsy or autopsy tissue sections confirms the fungal etiology of invasive infections [1, 5, 6, 13, 15, 19]. Tissue sections are usually stained with hematoxylin and eosin, but other staining techniques are also used in practice (e.g. Gomori-Grocott methenamine silver stain, periodic acid-Schiff stain) [2, 88, 89]. Histopathological tissue sections from a patient with invasive aspergillosis of the heart and lung are shown in Sulik-Tyszka et al. [89].

In tissue sections, *Aspergillus* appears as septate hyphae, with dichotomous branching at 45° angles suggestive of *Aspergillus* spp. [2, 19]. Conidiophores and fruiting bodies are rarely seen, except in areas exposed to air, for example, bronchi [19]. Invasive lesions are characterized by an area of necrosis and non-caseating

granulomatous inflammation. A characteristic feature of invasive aspergillosis is trespassing of the the fungus into the blood vessels, with subsequent infarction and tissue necrosis.

5.2 Direct microscopic examination, culture, and identification of *Aspergillus* spp.

Clinical samples for isolation of *Aspergillus* spp. in culture depend on the clinical symptoms and suspected localization of infection. In the diagnosis of invasive pulmonary aspergillosis, a lung biopsy or a sample of BALF is recommended. Other specimens, such as bronchial or endotracheal aspirates, pleural fluid, and also sputum may be cultured as well [5, 6, 19]. Blood cultures can be done, but are usually negative. In fact, any suspicious lesion (e.g. cutaneous or skeletal) should be biopsied and cultured for fungi [19].

The direct microscopic examination allows not only rapid detection of the fungus directly in the specimen, but also its preliminary identification.

A universal solid medium for culturing fungi is Sabouraud agar. When culturing a sample obtained from a site which is primarily nonsterile, Sabouraud agar supplemented with chloramphenicol and gentamicin is used. Czapek-Dox agar and 2% malt extract agar are also used, as well as liquid media [19]. Commercially available media are recommended in order to standardize the culture methods.

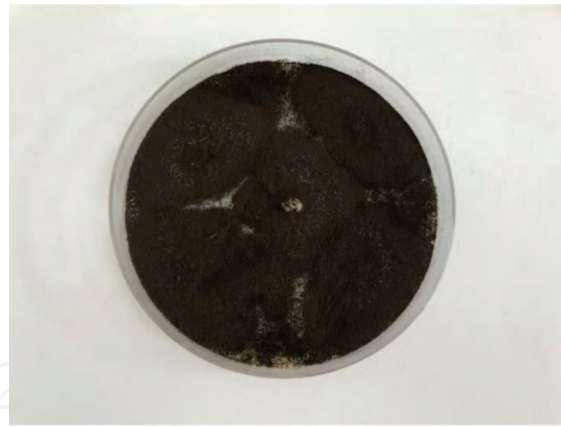
Identification of the isolates belonging to the genus *Aspergillus* relies on evaluation of the colony morphology and color (both on the upper and reverse sides of the agar plate), and diffusion of the pigment into the medium (**Figure 1**). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) technique may be used in the identification of *Aspergillus* spp. and is a promising tool, particularly for the identification of rare species of *Aspergillus* [90, 91]. This method allows a rapid and reliable identification of the isolate and therefore, an early start of effective antifungal therapy.

Interpretation of the mycological culture results is important and depends on the clinical picture [19]. In a routine microbiology laboratory work, most of the isolates of *Aspergillus fumigatus* do not indicate proven or probable infection. However, cultures positive for *Aspergillus fumigatus*, in the appropriate epidemiological and clinical setting, such as highly immunocompromised transplant recipients, are strongly associated with the presence or risk of IA and therefore should not be disregarded [19]. Also, the isolation of *Aspergillus fumigatus* in hematological patients, even from nonsterile samples, is generally regarded as potentially significant [19]. It has been reported that in heart transplant recipients with suspicion of invasive aspergillosis, a culture positive for *Aspergillus* has a positive predictive value (PPV) of 60–70% [92]. Higher PPV (78–91%) was linked to the isolation of *Aspergillus fumigatus*, with a further increase to 88–100% when *Aspergillus fumigatus* is recovered from a respiratory specimen other than sputum [92].

It should be emphasized that in transplant recipient's fungal cultures may be negative, despite disseminated infection, and in invasive aspergillosis, blood cultures are usually negative, even in patients with *Aspergillus* endocarditis [19].

5.3 Serological testing in the diagnosis of aspergillosis

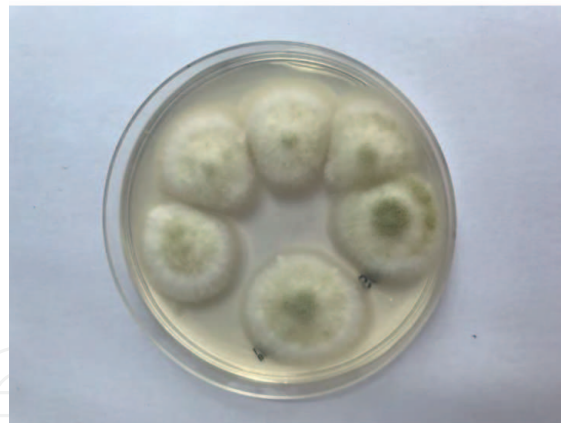
Serological tests are widely used in the laboratory diagnostics of aspergillosis. Conventional methods, such as culture of clinical samples and direct microscopy of the specimens, have low sensitivity and may give positive results in the late stages of the disease [19]. Furthermore, suitable specimens for these methods may be difficult to obtain in severely ill, immunocompromised patients. In recent years,



A



B



C

Figure 1.
Aspergillus spp. growth on Sabouraud agar. (A) Aspergillus niger; (B) Aspergillus fumigatus; (C) Aspergillus flavus.

serological techniques are being used increasingly on such specimens, like serum or BALF, for detection of fungal antigens (e.g. galactomannan, (1,3)- β -d-glucan) and anti-galactomannan antibodies against *Aspergillus* [44]. Detection of galactomannan in BALF or serum is at present recommended in the American and European guidelines on laboratory diagnosis of invasive aspergillosis in immunocompromised patients [5, 6, 13].

Duration of antifungal therapy may be guided not only by monitoring of galactomannan levels, but also by the clinical status of the patient and radiological findings.

5.3.1 Galactomannan (GM)

Galactomannan (GM) is a cell wall component of *Aspergillus* spp., released from *Aspergillus* hyphae, while they invade the host tissue, and therefore, it is a specific marker of this fungus [19]. This test may be helpful in early diagnosis of IA (median of 6 days before the symptoms appear) before the infection becomes disseminated [19]. Galactomannan can be detected with the use of latex tests (detection level 15 ng/ml) or more sensitive immunoenzymatic assays, in which 1 ng/ml of GM can be detected. Variation in sensitivity of these tests is being reported, which may due to the different cut-off values for a positive GM result in Europe (1.5 ng/mL) and the USA (0.5 ng/mL) [19].

Galactomannan concentration in the bronchoalveolar lavage fluid, in combination with other diagnostic tests (e.g. chest CT scan or mycological culture) is recommended as a test for the diagnosis of IA in lung and nonlung transplant recipients [71]. Galactomannan in BALF sample has proven superior to serum testing with high sensitivity (67–100%) and specificity rates (91–100%) for the diagnosis of invasive aspergillosis in lung transplant recipients [44]. In patients with prolonged neutropenia and allogeneic stem cell transplant recipients during the early engraftment phase, GM detection is commonly used, and serial screening for GM in serum has a high sensitivity and a negative predictive value for IA [13, 14]. However, serial screening for GM is not recommended in patients receiving antifungal prophylaxis with anti-*Aspergillus* spectrum of activity [13].

False-positive results of galactomannan detection have been documented in up to 13–29% of the liver transplant recipients and in 20% of the lung transplant recipients [44]. In the liver transplant recipients, false-positive results of galactomannan tests were more often seen in patients with transplantation for autoimmune liver disease, perioperative prophylaxis with β -lactam antibiotics, and requirement of dialysis. Most false-positive tests after lung transplantation occurred in the early post-transplant period: that is, in 43% within 3 days, in 64% within 7 days, and in 79% within 14 days of surgery. False-positive results of galactomannan detection are linked to several factors, such as antibiotic therapy with specific groups of antibacterials (such as some cephalosporins, carbapenems, amoxicillin-clavulanate, ampicillin/sulbactam, and piperacillin/tazobactam), cyclophosphamide therapy, as well as administration of blood products, albumin or immunoglobulins, or the use of cellulose hemodialysis membranes [93, 94].

5.3.2 (1,3)- β -D-glucan

Detection of (1,3)- β -D-glucan is a nonspecific test for fungal infection, as it is one of the main cell wall polysaccharide components of many fungi [19]. Available diagnostic tests are characterized by a high sensitivity and specificity and enable detection of (1,3)- β -D-glucan at the concentration of >1 pg/ml. It has been reported that in living-donor, liver allograft recipients, detection of (1,3)- β -D-glucan was useful for the diagnosis of invasive aspergillosis [95]. Several factors have been linked to false-positive results of the tests for (1,3)- β -D-glucan, which are similar to those for galactomannan detection [19].

5.4 Molecular methods

At present, molecular diagnostic tests for *Aspergillus* spp. are not available in routine clinical microbiology laboratories, however, in the near future, they will be used increasingly, particularly for identification of unusual species, with specific

profiles of susceptibility or resistance to antifungals [19, 44, 96]. Recently, PCR technique for detection of *Aspergillus* spp. has been extensively validated and will be included in the diagnostic criteria in the revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC-MSG) definitions [97]. A comprehensive review of the molecular diagnosis of invasive aspergillosis has recently been published [85].

The majority of molecular methods which can be used in the clinical microbiology laboratories are based on PCR technique. These methods are particularly useful for testing of lung specimens. It has been reported, that quantitative PCR test used for diagnosis of IA with a sample of bronchoalveolar lavage fluid was characterized by 67% sensitivity and 100% specificity [98]. Perhaps in the future, quantitative PCR tests will also be used to monitor the response to antifungal treatment in patients with IA [19].

Fluorescence in situ hybridization-based molecular method is a promising approach in the *A. fumigatus* detection in the tissues [87]. Species identification of *Aspergillus* isolates may be made by β -tubulin and calmodulin gene sequencing [91]. Further analyses are needed to evaluate the significance of the results of molecular tests in immunocompromised patients suspected of invasive aspergillosis.

6. Treatment of invasive aspergillosis

Successful treatment of invasive aspergillosis comprises early diagnosis of the disease, selection of an appropriate antifungal agent active against fungi of the genus *Aspergillus*, and prompt initiation of antifungal therapy, as well as surgical debridement, particularly in immunocompromised patients, such as HSCT and SOT recipients [3, 44]. Proper pharmacokinetics, pharmacoeconomics, and no interactions with other medications (e.g. immunosuppressants) administered to the patient are further factors which determine the choice of a proper antifungal agent.

At present, there are three groups of antifungal agents, which can be used in the therapy of aspergillosis: azoles, polyenes, and echinocandins [99]. The choice of treatment regimen depends on several factors, including the host's immune status, liver, and kidney functions, and prior antifungal therapies [99]. Treatment regimens of invasive aspergillosis are shown in **Figure 2**.

It should be emphasized that in the choice of proper antifungal therapy, susceptibility testing of the cultured isolate is important in view of an emerging resistance of some *Aspergillus* strains to azoles, as well as identification to the species level, as resistance to antifungals is more likely with certain species of *Aspergillus* other than *Aspergillus fumigatus* [99]. For example, *A. terreus* has a high minimum inhibitory concentration (MIC) to amphotericin B, while *A. calidoustus* has to numerous antifungals [99]. The role of a combination antifungal treatment for primary therapy of IA remains controversial; however, it may be considered (e.g. voriconazole with an echinocandin) for treatment of infection caused by these species [71, 99].

Apart from antifungal therapy, surgery may be indicated in patients with invasive aspergillosis. It applies to the cases in which the disease is localized and infection site is easily accessible to debridement (e.g. invasive fungal sinusitis or localized cutaneous lesions) [5, 6]. Surgical excision or debridement may be used for both diagnostic and therapeutic purposes [44].

Surgery of the sinuses may involve removal of the granulation tissue and necrotic bone [3]. Surgery may also be required in patients with endocarditis, osteomyelitis, or focal lesions in the central nervous system [5, 6]. Surgery is particularly indicated for persistent, or a life-threatening hemoptysis, lesions in the proximity

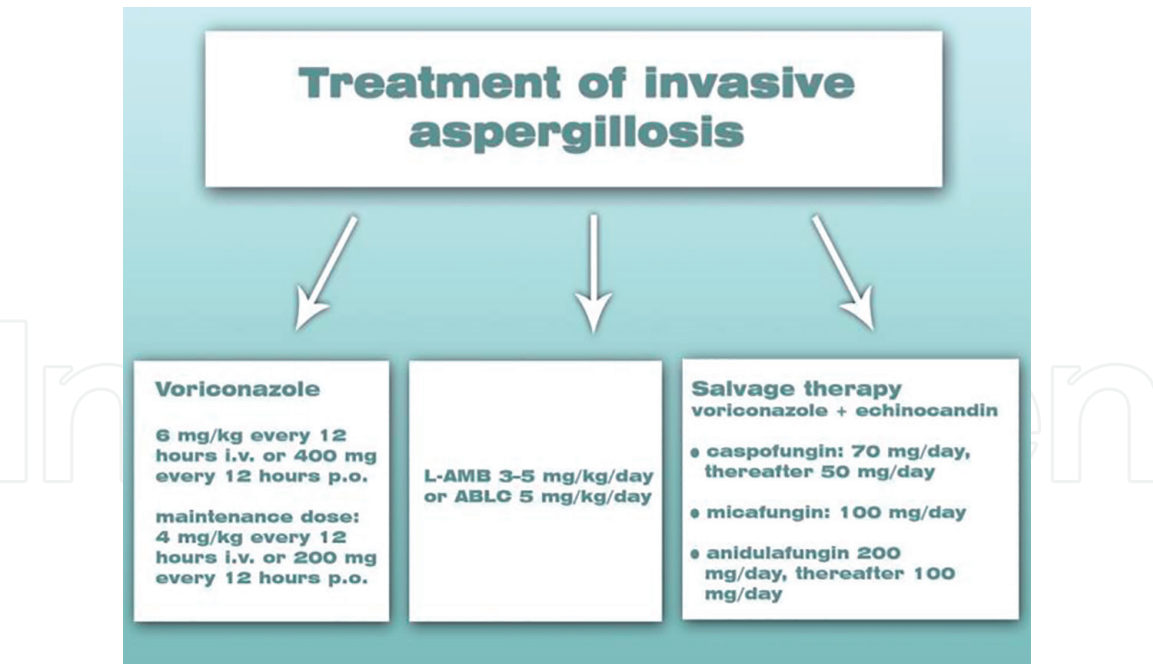


Figure 2.
Treatment of invasive aspergillosis.

of great vessels or pericardium, nasal and sinus infections, single cavitary lesion in the lung, intracranial abscesses, as well as lesions invading the pericardium, bone, the subcutaneous, or thoracic tissue [100].

6.1 Triazoles

According to the newest guidelines of ECIL-6 (the European Conference on Infections in Leukemia), recommendations of Infectious Diseases Society of America (IDSA) and European Society of Clinical Microbiology and Infectious Diseases, European Confederation of Medical Mycology, European Respiratory Society (ESCMID/ECMM/ERS) as well as expert groups, the drugs of first choice in therapy of invasive aspergillosis are triazoles—voriconazole or isavuconazole in monotherapy [5, 6, 13, 20, 71].

Isavuconazole is as effective as voriconazole, and in addition, it is characterized by a better safety profile, therefore in the newest guidelines, it has been granted AI recommendation equally with voriconazole [5, 6]. In patients who do not tolerate voriconazole, therapy with itraconazole or posaconazole may be considered; however, cross-resistance between azoles may be a problem [5]. An alternative antifungal agent used in the therapy of this disease is liposomal amphotericin B [5, 6, 13, 71]. It is emphasized that the chosen agent should be implemented as quickly as possible after fungal etiology of the infection has been suspected [5, 15]. At present, the routine use of combination therapy is neither recommended as a first line treatment, nor the use of echinocandins as the primary treatment [5].

In recent years, an increase in the percentage of strains of *Aspergillus fumigatus* resistant to azoles (including voriconazole) is being observed, which may pose a therapeutic problem [20, 101–103]. Resistance is mainly due to mutations in the gene *cyp51A* and/or overexpression of the efflux pumps in the cells of these fungi [101–106]. Lestrade et al. showed that among 196 patients with IA, in 37 (19%) infection was caused by a strain of *A. fumigatus* resistant to voriconazole, which was linked to >20% increase in the mortality rate in this group of patients in comparison to individuals who received proper antifungal treatment [107]. In 2006–2016, Borman et al. analyzed 2501 clinical strains of *Aspergillus fumigatus*, among which

3.1% were resistant to voriconazole and 12.5% were resistant to amphotericin B [108]. The range of MIC values for these strains to voriconazole was 0.03–16.0 µg/ml and to amphotericin B was 0.06–4.0 µg/ml [108]. In a study comprising 105 clinical strains of *Aspergillus fumigatus*, a significant difference was reported in the percentage of strains resistant to triazoles among the isolates cultured from samples obtained from hematological patients (15.9%), in comparison with the group of patients treated in the ICU (4.5% strains) [101].

Voriconazole has emerged as the preferred agent for primary therapy of IA. Its efficacy has been confirmed in many studies, including hematopoietic stem cell transplant recipients and patients with hematological malignancies, as well as SOT recipients [44]. Voriconazole was effective in heart transplant recipients, in SOT recipients with central nervous system aspergillosis. In a lung transplant patient with *Aspergillus* endophthalmitis, voriconazole has been used in the form of an intravitreal injection [44].

6.2 Liposomal amphotericin B

An alternative antifungal agent recommended in therapy of IA is liposomal amphotericin B (L-AmB), which is active *in vitro* against the majority of strains of *Aspergillus* [5, 6, 13, 44, 71, 99]. However, it should be remembered that some species of *Aspergillus* (e.g. *A. terreus*) neutropaenic patients may be resistant to this antifungal agent.

6.3 Echinocandins

Echinocandins (caspofungin, micafungin, or anidulafungin) are not recommended as first-line therapy of invasive aspergillosis, as they exhibit only fungistatic (not fungicidal) activity against the isolates of *Aspergillus* spp. They can be considered in salvage therapy; however, in combination with voriconazole, isavuconazole, or liposomal amphotericin B [44, 99, 109].

6.4 Salvage therapy

In patients not responding to monotherapy with antifungal agents recommended as first-line therapy, such as voriconazole, isavuconazole, or liposomal amphotericin B, a salvage therapy must be considered, with the use of a combination antifungal regimen [99]. In these cases, it is suggested to combine an echinocandin (caspofungin, micafungin, or anidulafungin) with voriconazole, isavuconazole, or liposomal amphotericin B, while there are no clinical data to support the use of triazoles in combination with amphotericin B [99]. Apart from salvage antifungal therapy, reduction of the doses of immunosuppressive agents (if feasible), as well as surgery should be considered in these patients.

6.5 Duration of antifungal therapy

The duration of therapy for IA is usually 12 weeks, but may range from 3 to >50 weeks or may be even lifelong [5, 6, 13, 44]. Many factors may influence it, such as the response to administered therapy, the patient's immune status and underlying diseases [44]. It is recommended to continue therapy until all clinical and radiographic abnormalities have resolved, and cultures are negative for *Aspergillus*. In transplant recipients, it is important to lower the doses of immunosuppressive agents, as well as to monitor an allograft function [44]. Patterson et al. and other expert groups recommend that therapy of invasive pulmonary aspergillosis should be continued for at least 6–12 weeks, depending on the site of disease, degree and duration of immunosuppression, and evidence of improvement of the patient's clinical status [5, 6]. In patients

with stable and pharmacokinetically predictable status, physicians should consider switching from intravenous to oral therapy [13]. If immunosuppression has to be continued after successful therapy of invasive aspergillosis, secondary prophylaxis should be initiated to prevent recurrence of the infection [5, 6].

6.6 Prophylaxis of invasive aspergillosis in transplant recipients

Antifungal prophylaxis against aspergillosis should be used in patients at high risk of IA during prolonged neutropenia [5, 6]. It is recommended to administer posaconazole, voriconazole, or micafungin (caspofungin is also probably effective) [5, 6]. Prophylaxis with itraconazole is effective, but absorption and tolerability of this drug may be a problem.

For allogeneic HSCT recipients with graft-versus-host disease (GVHD), who are at high risk for IA, prophylaxis with posaconazole is recommended, but other azoles active against *Aspergillus* may also be used [5, 6]. In patients with chronic immunosuppression associated with GVHD antifungal prophylaxis should be continued throughout the duration of immunosuppression [5, 6].

According to the ECIL-6 and other recommendations, antifungal prophylaxis with either a systemic triazole (voriconazole or itraconazole) or an inhaled AmB product is recommended for 3–4 months after lung transplantation [5, 6]. Aerosolized amphotericin B is an option which allows the direct administration of the antifungal agent into the transplanted lung, with avoidance of systemic unwanted effects and drug–drug interactions [44]. However, for certain groups of lung transplant recipients (single lung transplant recipients, mold colonization before or after lung transplantation, mold infections detected in explanted lungs, and fungal infections of the sinus) systemic voriconazole or itraconazole is recommended rather than inhaled AmB. Patterson et al. and other experts recommend reinitiation of antifungal prophylaxis in lung transplant recipients who receive immunosuppression augmentation with thymoglobulin, alemtuzumab, or high-dose corticosteroids [5, 6].

For other SOT recipients, antifungal prophylaxis against IA is not routinely recommended and should be based on the institutional epidemiology of aspergillosis and assessment of the patient's risk factors [5, 6, 44]. A common approach to antifungal prophylaxis in liver transplant recipients is to target high-risk patients (fulminant hepatic failure, reoperation, retransplantation, or with renal failure), and it is administered during pre-transplant hospitalization and for the first-month posttransplant. Risk factors for IA have also been identified in heart transplant recipients, such as pretransplant colonization with *Aspergillus* spp., reoperation, cytomegalovirus (CMV) infection, and renal dysfunction. Other risk factors for IA, which may justify antifungal prophylaxis are institutional outbreaks and prolonged or high-dose corticosteroid therapy; however, the optimal duration of such prophylaxis has not been determined [5, 6].

6.7 Immunomodulatory agents and new therapeutic options

At present, it is recommended to reduce the doses of immunosuppressive therapy administered to the patient (or eliminate it, if possible), as this improves the outcome of anti-*Aspergillus* therapy [5, 6]. Other approaches can be considered in cases not responding to standard antifungal therapy, such as granulocyte transfusions in neutropenic patients with IA, or recombinant interferon- γ as prophylaxis in patients with chronic granulomatous disease (CGD) [5, 6].

A relatively new approach to the therapy of invasive aspergillosis in immunocompromised patients involves the use of immunomodulatory agents which would enhance the host's immune system [44]. There is a potential for clinical use of

selected cytokines or colony-stimulating factors (e.g. granulocyte colony-stimulating factor, G-CSF; granulocyte-macrophage colony-stimulating factor, GM-CSF; interferon- γ) with immunomodulatory effect [44, 110].

There is an ongoing search for new, more effective antifungals, which will be active also against drug-resistant isolates of *Aspergillus* spp. Currently, there are new classes of antifungal drugs under development—two agents in phase 2 study for the therapy of systemic invasive fungal infections and one drug in phase 1 [111]. Among them are agents which show activity against resistant to azoles *cyp51A* mutants of *Aspergillus* spp.

7. Summary

Diagnosis of invasive aspergillosis remains a challenge for clinicians and microbiologists. Progress in modern diagnostic methods and imaging techniques may contribute to an early and reliable diagnosis of infections caused by *Aspergillus* spp. This is particularly important in immunocompromised patients, such as HSCT and SOT recipients. Proper choice and early commencement of antifungal therapy increase the chances for survival and recovery of these patients from invasive aspergillosis.

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